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Trophic state changes can affect the importance of methane-derived carbon in aquatic food webs

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Abstract

Methane-derived carbon, incorporated by methane oxidizing bacteria, has been identified as a significant source of carbon in food webs of many lakes. By measuring the stable carbon isotopic composition ($\delta^{13}C$ values) of particulate organic matter, Chironomidae, and *Daphnia* spp. and their resting eggs (ephippia) we show that methane-derived carbon presently plays a relevant role in the food web of hypertrophic Lake De Waay, the Netherlands. Sediment geochemistry, diatom analyses and $\delta^{13}C$ measurements of chironomid and *Daphnia* remains in the lake sediments indicate that oligotrophication and re-eutrophication of the lake during the 20th century had a strong impact on in-lake oxygen availability. This, in turn, influenced the relevance of methane-derived carbon in the diet of aquatic invertebrates. Our results show that, contrary to expectations, methane-derived relative to photosynthetically produced organic carbon became more relevant for at least some invertebrates during periods with higher nutrient availability for algal growth, indicating a proportionally higher utilization of methane-derived carbon in the lake´s food web during peak eutrophication phases. Contributions of methane-derived carbon to the diet of the investigated invertebrates are estimated to have ranged from 0-11 % during the phase with the lowest nutrient availability to 13-20 % during the peak eutrophication phase.

Introduction

Eutrophication of inland waters as a consequence of human impact has a detrimental effect on different aspects of the water quality of lakes, rivers and streams [1]. For example, the process can change the chemical properties of the water, leading to oxygen depletion [2] and accumulation of nutrients in the anoxic hypolimnion [3]. Lake ecosystems with low oxygen concentrations and high nutrient loading are characterized by higher output of the important greenhouse gas methane (CH_4) than oxygen-rich lakes and lakes with lower nutrient

availability [4,5], particularly via gas bubbles (ebullition) and release of CH_4 stored in the anoxic hypolimnion during lake overturning [6]. CH_4 formed in lakes can be oxidized by methane-oxidizing bacteria (MOB), predominantly in oxygenated sections of the lake basin [7]. Biogenic CH_4 in freshwater systems is characterized by distinctly low ratios between the stable carbon isotopes ^{13}C and ^{12}C (expressed as $\delta^{13}C$ values; -80 to -50 ‰) [8,9] and MOB are known to discriminate against the heavier ^{13}C when metabolizing CH_4 resulting in even lower $\delta^{13}C$ values of MOB biomass [10]. These very low values do not occur in aquatic and terrestrial photosynthetic primary producers (-35 to -10 ‰ [11–15]). The very low observed $\delta^{13}C$ values of e.g. larvae of non-biting midges (Chironomidae) of the tribe Chironomini [16,17] and planktonic water fleas of the genus *Daphnia* (Cladocera) [18] in some lakes are therefore considered a clear indication of MOB, or organisms feeding on MOB, forming a relevant part of the diet of these organisms.

Planktonic filterers such as *Daphnia* can graze MOB from the water column during stratification, a process which can effectively reduce MOB biomass and lead to increased epilimnetic CH_4 concentrations at least in some shallow boreal lakes [19]. Furthermore, *Daphnia* has been shown to rely strongly on MOB-derived carbon during autumn overturning, when the CH_4 stored in deep anoxic water layers of stratified lakes comes into contact with oxygen [20]. Benthic invertebrates that can incorporate CH_4-derived carbon, such as chironomid larvae of the tribe Chironomini, either feed on MOB in the sediments (deposit feeders) or MOB associated with suspended organic particles (filter feeders). Some Chironomini larvae have have been shown to actively maintain an oxic-anoxic interface within their tubes, providing a habitat for MOB which they feed on [21]. $\delta^{13}C$ values of chironomids and *Daphnia* closely reflect those of their food source (differences of 0-1 ‰) and of their fossilizing chitinous structures (reported offsets of 0-1 ‰) [22–25]. Chitinous remains deposited and buried in the lake sediments retain their original isotopic composition [23]. Hence, analysis of $\delta^{13}C$ values of “fossil” chironomid and *Daphnia* remains can provide

78 insights on their past food sources and into whether CH_4-derived carbon formed a major
79 component of their diet [23,26–31]. Available studies indicate that chitinous remains with low
80 $\delta^{13}C$ values are deposited in lakes with high surface and deep water CH_4 concentrations and
81 diffusive CH_4 emissions [28,29].

82 It is unclear whether an increase in nutrient loading, in addition to a higher CH_4
83 output, also results in a higher contribution of CH_4-derived carbon to the lake food webs.
84 Higher photosynthetic primary productivity associated with higher nutrient loading can
85 increase the availability of algal organic matter. As a consequence, a higher proportion of
86 algae in the diet of invertebrate groups that may also incorporate CH_4-derived carbon could be
87 expected. However, higher algal productivity can also lead to decreased oxygen
88 concentrations in lake sediments and deep water layers, and associated increases in CH_4
89 production and CH_4 availability in lake ecosystems. This can favour the growth of MOB and
90 their temporal and spatial availability within lakes. Therefore, how the relevance of CH_4-
91 derived carbon in the food web of lakes changes under influence of (past or future) changes in
92 nutrient concentrations and productivity remains poorly constrained, particularly on decadal
93 time scales which are not covered by instrumental measurements of CH_4 concentrations and
94 $\delta^{13}C$ values in aquatic ecosystems.

95 The $\delta^{13}C$ values of chitinous remains of aquatic invertebrates were previously mainly
96 studied in oligo- to mesotrophic, often remote lakes (e.g. [27,29,30,32]). Here, we present a
97 study of the $\delta^{13}C$ values of fossil Chironomini head capsules and *Daphnia* resting eggs
98 (ephippia) from recent (20^{th} century) sediments from a small and presently hypertrophic
99 dimictic lake in the Netherlands (Figure 1). The study lake, Lake De Waay, underwent a
100 transition from eutrophic (TP ~100 µg l^{-1}) to more mesotrophic conditions (TP ~40 µg l^{-1}) and
101 then again to hypertrophic conditions (TP >100 µg l^{-1}) during the past ~100 years [33,34].
102 The impact of 20^{th} century temperature changes on lake ecosystems in the Netherlands is

likely to have been relatively minor compared to direct anthropogenic environmental disturbances. Therefore, this study provides an opportunity to investigate the effects of both oligotrophication and (re-)eutrophication and the associated changes in oxygenation regime on the contribution of CH_4-derived carbon to the aquatic food web, and indirectly on in-lake dissolved CH_4 availability, under relatively stable climatic conditions. We compare fossil invertebrate $\delta^{13}C$ values with a diatom-inferred reconstruction of total phosphorus concentrations (DI-TP) in the lake water and the Fe : Mn ratio of the sediments, which is expected to increase with decreasing oxygen availability at the sediment-water interface [35]. If CH_4-derived carbon became more relevant for Lake De Waay's foodweb under conditions with higher nutrient availability we expect to see a positive relationship between nutrient availability (DI-TP) and anoxia (Fe : Mn ratio), which in turn are expected to be negatively related to $\delta^{13}C$ values of the examined invertebrate groups that can incorporate CH_4-derived carbon. Conversely, if higher availability of algal material in the eu- to hypertrophic phases led to a lower relevance of CH_4-derived carbon for the investigated aquatic invertebrates, we expect to see positive relationships between the $\delta^{13}C$ values of the studied invertebrate groups and DI-TP and Fe : Mn in Lake De Waay.

Methods

Current conditions in the lake

To assess the current range of $\delta^{13}C$ values of chironomids, *Daphnia*, floating *Daphnia* ephippia, and suspended particulate organic matter (POM) in the water column of Lake De Waay, field sampling was conducted on September 3 and November 30 2009, and March 1 and June 1 2010. Living organisms were collected using plankton nets, kicknets and inspection of submerged wood and rope. In the lab, organisms were sorted and transferred to

127 tin cups and water was filtered for $\delta^{13}C$ analysis (see supplementary material for details on
128 sampling and processing).

129 On September 3 2009, nine sediment cores were taken using a gravity corer
130 (UWITEC, Austria): five at 14.5 m water depth and four at 5 m water depth. The top 2 cm of
131 sediment were sampled in the field. Sediment was treated with 10% KOH for 2 hours at room
132 temperature and sieved (200 μm). From the sieve residue approximately 50 subfossil *Daphnia*
133 ephippia and head capsules of chironomids of the tribe Chironomini were cleaned with a
134 forceps and placed in pre-weighed tin cups for isotope analysis, resulting in one sample for
135 $\delta^{13}C$ analysis of *Daphnia* ephippia from 14.5 m and two from 5 m water depth, and two
136 samples of Chironomini head capsules from 5 m water depth.

137 On August 9 2011, water samples were collected to characterize CH_4 concentration in
138 the lake water during late summer stratification, and for analysis of $\delta^{13}C$ values of CH_4 as a
139 basis for isotope mixing models (see [36] and the supplementary material for full details). In
140 addition, we retrieved more surface sediments (0-2 cm) from 2.5, 8, and 14.5 m depth as
141 described above which were sent to the Netherlands Institute of Ecology (NIOO) for
142 quantitative polymerase chain reaction (qPCR) analysis to assess the presence and abundance
143 of methanogens and MOB (see supplementary material for details on the methods).

144

145 *Down-core study*

146 On November 30 2009, a 68 cm long core (WAY09) was taken at 8 m water depth (just
147 beneath the summer thermocline) using a gravity corer, and sampled on site at 2 cm intervals.
148 17 samples were prepared for gamma spectrometric determination of ^{137}Cs (see
149 supplementary material). A subsample from the same sampling depths (~12 mg dry weight)
150 was treated with 2.5 % HCl to remove carbonates [35], then freeze dried and subsequently

loaded into tin cups for bulk sediment $\delta^{13}C$ analysis. Further subsamples were used to reconstruct diatom-inferred total phosphorus concentrations (DI-TP) following Kirilova et al. [33,34] (see supplementary material).

Sediment cores taken by Kirilova et al. [34] from Lake De Waay (WAY05) were previously analyzed using a XRF core scanner (Avaatech, the Netherlands). Here we use the ratio between Fe and Mn as an indication of past changes in the oxygen regime of the lake. Higher values in this ratio are indicative for lower oxygen availability [37,38]. Cores WAY09 (this study) and WAY05 were correlated by comparing their ^{137}Cs profiles (see supplementary material). Invertebrate remains were sorted from 17 samples and analysed for their $\delta^{13}C$ values, following the same procedure as for subfossil remains in the surface sediment samples (see above and supplementary material). Relationships and lags between records were quantified by cross-correlation analysis, after linear detrending of the time series (see supplementary material).

To assess how much carbon in the diet of Chironomini and *Daphnia* could derive from CH_4, and how strongly this contribution may have varied in the past, we applied a two-source mixing model [39] to the modern and down-core invertebrate $\delta^{13}C$ data. As end-members, $\delta^{13}C$ values of POM (average) and a CH_4 sample from the sediment pore space were chosen, the latter modified by -6 ‰ to account for fractionation by MOB [10] (see supplementary material for further details). For the mixing model we assumed no change in baseline $\delta^{13}C$ of CH_4 and POM over time because $\delta^{13}C$ values of bulk sedimentary organic matter remained very stable within our record (-30.5 ± 0.5 ‰).

Results

Current conditions in the lake

175 POM $\delta^{13}C$ values (both 0-60 and 0-250 µm fractions were analysed) were around -36 ‰ in
176 late fall and late winter, and -30 ‰ in late spring and late summer (Figure 2A). The $\delta^{13}C$
177 value of bulk sedimentary organic matter in the top sediment layer at the coring site was -31.3
178 ‰ and the atomic C : N ratio was 13.1, suggesting a predominantly lacustrine origin of
179 organic material with some terrestrial contributions [35]. Chironomids of the tribe
180 Chironomini (Figure 2B) had an average $\delta^{13}C$ value of -32.5 ‰ (n = 31, standard deviation
181 (SD) ± 1.95 ‰), which agrees well with values of Chironomini head capsules from the
182 surface sediment samples (-33.0 and -33.4 ‰). Only three individual chironomid larvae had
183 lower $\delta^{13}C$ values than the POM, all belonging to *Glyptotendipes barbipes*-type and collected
184 in late fall (-37.8, -39.0 and -39.0 ‰). *Daphnia* $\delta^{13}C$ values were highly variable (average -
185 36.6 ± 6.8 ‰, n = 6; Figure 2C). In late winter they were much lower than the POM (-44.2
186 and -44.3 ‰) as opposed to late summer (-26.5 ‰) and late spring (-35.0, -34.6 and -34.8 ‰).
187 Insufficient *Daphnia* were collected in late fall for a measurement. Floating *Daphnia* ephippia
188 $\delta^{13}C$ values were lower (average -41.7 ± 4.8 ‰, n = 9; Figure 2C) than POM throughout the
189 year and the values were in agreement with those found in ephippia from surface sediments (-
190 38.6, -39.3 and -39.6 ‰). Most notably, in late fall the floating ephippia reached $\delta^{13}C$ values
191 as low as -49.4 ‰.

192 $\delta^{13}C$ values of six CH_4 samples from anoxic waters and sediments ranged from -69.0
193 to -67.5 ‰, whereas values for two samples from oxygen-rich waters were -51.3 and -50.6 ‰
194 (Figure 2D). CH_4 concentrations in the surface and bottom waters were 1.1 and 479 µM,
195 respectively. QPCR analyses revealed that gene copy numbers of methanotrophic bacteria as
196 well as of methanogenic archaea in sediment samples increased with water depth
197 (supplementary Table 1). At 2.5 m water depth the numbers of methanotrophs in the
198 sediments were below detection limit (~ 10^3 gene copies gram sediment^{-1}). However, a nested
199 PCR approach indicated that MOB were present but below detection of qPCR assays. Type Ia
200 MOB dominated the methanotrophic community while type II MOB could not be detected.

Down-core study

Trophic history and oxygen availability

Fossil diatom assemblages (presented in the supplementary material) indicate clear shifts in TP in Lake De Waay in the past ca. 100 years. The DI-TP values for Lake De Waay suggest hypertrophic conditions (>100 µg TP l^{-1}, Figure 3) in the lower section of the core. This is followed by a phase until ca. 1955 with a drop in DI-TP to around 35 µg l^{-1} which is related to hydrological changes in the lake's catchment as a result of surface water management [34]. DI-TP increases again gradually between ca. 1955 and 1975 to 100 µg l^{-1} as the lake underwent a distinct re-eutrophication. From thereon, the lake reverted back to hypertrophic conditions as DI-TP exceeds 100 µg l^{-1}, which is confirmed by water column TP measurements in 2011 (Figure 1; [36]).

The oligotrophication during the early 20th century is associated with a distinct lowering of the Fe : Mn ratio from 15 to 7 in core WAY05 (Figure 3). This suggests an increase in oxygen availability at the sediment-water interface in the centre of the lake [37,38]. The last part of the 20th century is then characterized by increasing Fe : Mn to values around 15 indicating more anoxic conditions at the sediment-water interface. Both trends in Fe : Mn follow the oligo- and eutrophication of the lake as inferred by diatoms. Cross-correlation analysis revealed maximum correlations between DI-TP and Fe : Mn, if the records are shifted by 1-2 sample steps (ca. 5 - 10 years; correlation coefficients 0.55 and 0.56, respectively), suggesting that the response of lake oxygenation lags ca. 10 years behind the changes in trophic state (supplementary Figure 3).

Down-core $\delta^{13}C$

225 Bulk organic matter in the sediments had an average $\delta^{13}C$ value of -30.5 ‰, with little change
226 (SD ± 0.5 ‰) throughout core WAY09 (Figure 4). Before ca. 1940 and after ca. 2000 head
227 capsules of the Chironomini had low $\delta^{13}C$ values (-35 ‰ and lower). Clearly higher values
228 are recorded between ca. 1940 and 2000 (-33.8 to -31.2 ‰). *Daphnia* ephippia $\delta^{13}C$ values
229 rise gradually from -40.0 to -33.7 ‰ between ca. 1920 and ca. 1970, after which a strong
230 opposite trend is apparent, with *Daphnia* $\delta^{13}C$ reaching values as low as -41.5 ‰ just below
231 the sediment surface. Cross correlation indicates strong negative correlations between Fe : Mn
232 and invertebrate $\delta^{13}C$ values at lags of 0 - 2 sample steps (ca. 0-10 years; correlation
233 coefficients -0.52 to -0.80) for *Daphnia* and 1-2 sample steps (ca. 5 - 10 years; correlation
234 coefficients -0.58 to -0.70) for Chironomini (supplementary Figure 3). The strongest negative
235 relationships were found for both invertebrate groups for a lag of 2 sample steps (ca. 10
236 years).

237

238 **Discussion**

239 *Current conditions in the lake*

240 Stable carbon isotope analysis of the different organisms and sedimentary remains in Lake De
241 Waay provided evidence for both photosynthetically produced and CH_4-derived carbon
242 contributing to the aquatic food web. POM $\delta^{13}C$ values in Lake De Waay (-36 to -30 ‰) were
243 in the range of POM collected in small, eutrophic and/or high DIC lakes and characteristic for
244 algal biomass (-39 to -18 ‰ [13,40,41]). $\delta^{13}C$ values of *Daphnia* in late winter (-44.3 ‰) and
245 floating *Daphnia* ephippia in late fall (-49.4 ‰) in Lake De Waay were clearly lower than
246 reported for photoautotrophic biomass in small eutrophic lakes, and distinctly lower than the
247 $\delta^{13}C$ values of water column POM we observed. Low $\delta^{13}C$ values of zooplankton, and
248 *Daphnia* in particular, have been linked to the uptake of CH_4-derived carbon [18,42]. The
249 pronounced difference in $\delta^{13}C$ values (~ -19 ‰) between CH_4 sampled in the sediment and in

250 the oxic surface waters is an indication of MOB activity within the lake, as preferential uptake
251 of $^{12}CH_4$ by MOB [10] leads to higher $\delta^{13}C$ values of the CH_4 pool. This is supported by the
252 qPCR analysis that indicated presence of DNA of MOB type I in the surface sediments. The
253 low $\delta^{13}C$ values we found in *Daphnia* and their ephippia confirm that these organisms
254 incorporate MOB-derived carbon in Lake De Waay.

255 Temperature, starvation and lipid content can influence invertebrate $\delta^{13}C$ values, but
256 these effects are typically small (± 0 to 2 ‰ [11,25,43,44]) compared to the shifts we
257 observed, indicating that seasonal variations in $\delta^{13}C$ values of *Daphnia* in De Waay mainly
258 reflect changing availability and $\delta^{13}C$ values of available food sources. Based on the two-
259 source mixing model, we estimate a contribution of CH_4-derived carbon to the diet of
260 *Daphnia* ranging from 0 % (in late spring and summer) to 27 % (based on body tissue) and 39
261 % (based on ephippia) in late fall and winter. This is in agreement with findings by Taipale et
262 al. [20], who found the strongest contribution of CH_4-derived carbon to the diet of *Daphnia* in
263 a polyhumic boreal lake in Finland in fall. Similarly, Harrod and Grey [45] and Morlock et al.
264 [46] reported Cladoceran $\delta^{13}C$ values 10-20 ‰ lower in fall and winter than in summer in
265 eutrophic lakes in Germany and Switzerland, respectively. These results indicate that these
266 invertebrates can utilise a CH_4-derived carbon source when the preferred food sources are less
267 readily available.

268 The Chironomini larvae were sampled in the littoral zone, whereas MOB-feeding
269 chironomids are mostly found in sediment exposed to low oxygen concentrations [47–49].
270 The sampling location may explain why the majority of the living Chironomini we sampled
271 did not exhibit as low $\delta^{13}C$ values as in some studies (e.g. [47]), even though CH_4 and MOB
272 appear to play a major role in the lake food web. Nevertheless, several individuals had $\delta^{13}C$
273 values distinctly lower than observed for POM and the other Chironomini larvae, suggesting
274 that CH_4-derived carbon may have contributed to their diet even in littoral habitats (12 to 15

% based on the mixing model). Agasild et al. [49] also reported at least 40 % CH_4-derived carbon in the diet of *Chironomus plumosus* found in the littoral, macrophyte-covered zone of a shallow lake.

Chironomini head capsules and *Daphnia* ephippia in the uppermost analysed sample in core WAY09 were also characterized by low $\delta^{13}C$ values (-38.5 ‰ for Chironomini and -41.5 ‰ for *Daphnia* ephippia), values which are again well below the average of modern water column POM and the sedimentary organic matter in the surface sediments. This suggests that the imprint of CH_4-derived carbon on the $\delta^{13}C$ values of the organisms is registered in the fossil record, even though this record integrates seasonal and spatial variability in *Daphnia* and chironomid $\delta^{13}C$ values.

Carbon sources during changing nutrient levels

The DI-TP reconstruction confirms, with higher temporal resolution, the conclusions by Kirilova et al. [33,34] that the lake went from eutrophic conditions at the beginning of the 20^{th} century to more mesotrophic conditions between ca. 1925 and 1955, followed by a trend to the current, hypertrophic conditions (Figure 3). The Fe : Mn record indicates lower availability of oxygen at the sediment-water interface during the eutrophic and hypertrophic phases (Figure 3), conditions that promote methanogenesis [50], although the variations in oxygen availability take place more gradually and lag those in nutrient concentrations by ca. 5-10 years. This lag may reflect the time needed to accumulate organic rich, oxygen-demanding sediments during eutrophication and the lingering oxygen demand of such sediments after oligotrophication [51]. The oldest and the most recent sediments, representing the highest nutrient levels and lowest oxygen availability, featured $\delta^{13}C$ values in chitinous remains of *Daphnia* and Chironomini that resemble the low values we found during the field survey (Figure 2; Figure 3). As discussed above, this suggests a contribution of CH_4-derived

carbon to the diet of *Daphnia* (up to 20 % based on the mixing model, Figure 3) and Chironomini (up to 12 %). In intermediate sections of the record, the analyzed invertebrate remains had distinctly higher $\delta^{13}C$ values, which may indicate a lower (or even a lack of) contribution of CH_4-derived carbon to the diets of *Daphnia* (less than 10 %) and Chironomini (less than 2 %) during this period. Variations in $\delta^{13}C$ values of *Daphnia* were more gradual than variations in Chironomini $\delta^{13}C$ values. However, the maxima in both curves closely followed the observed minimum in Fe : Mn values, with the strongest negative relationships between the records observed for a small lag of ca. 5 - 10 years (Supplemetary Figure 3). This suggests that variations in invertebrate $\delta^{13}C$ values were related to changes in oxygen availability in the hypolimnion resulting from changes in lake productivity.

There are alternative explanations for changes in invertebrate $\delta^{13}C$ values in lake sediment records. However, these cannot explain the full range of invertebrate $\delta^{13}C$ values observed for lake De Waay. $\delta^{13}C$ values of autochthonous photoautotrophic primary production may vary in lakes, due to changing ^{12}C-preference of algae during carbon uptake, and/or shifts in baseline $\delta^{13}C$ values of DIC. Lower algal growth rates under lower nutrient availability lead to higher discrimination against ^{13}C during photosynthesis and therefore more ^{13}C-depleted algal biomass (e.g. [52]). Therefore, this mechanism would have caused lower $\delta^{13}C$ values of algal biomass and correspondingly lower *Daphnia* $\delta^{13}C$ values during the mesotrophic conditions reconstructed for the lake ca. 1925-1955. This implies that a major increase in baseline $\delta^{13}C$ values of DIC would have been necessary to explain the increase in *Daphnia* $\delta^{13}C$ values, even exceeding the 8 ‰ shift observed in *Daphnia* ephippia. Considering the present DIC $\delta^{13}C$ values of -9.1 ‰ [28] this would only be possible if DIC reached unrealistically high $\delta^{13}C$ values of ~0 ‰, which excede the range of DIC $\delta^{13}C$ values reported in a wide range of lakes (-31.1 to -2.1 ‰, [53]).

Heterotrophic respiration of DOC can also lead to ^{13}C-depletion of DIC available to algae and consequently of organisms that feed on them, a process often reported for lakes with high DOC concentrations [54]. Since Lake De Waay is presently characterized by relatively low DOC concentrations (0.5 mmol l^{-1}; [55]), a strong increase in heterotrophic respiration of DOC during the second half of the 20th century is unlikely. Moreover, we would expect that major variations in $\delta^{13}C$ values of of algal production in Lake De Waay would have led to distinct variations in $\delta^{13}C$ values of bulk organic matter in the sediments of Lake De Waay.

As indicated above, factors such as starvation, temperature and lipid composition have only minor effects on $\delta^{13}C$ values of aquatic invertebrates (± 0 - 2 ‰) [11,25,43,44]. These factors therefore cannot (fully) explain the major changes in fossil invertebrate $\delta^{13}C$ values observed in De Waay. Finally, changes in the timing of *Daphnia* ephippia production may potentially have some effect on the $\delta^{13}C$ values of the fossil assemblage. However, given the supporting evidence of changes in trophic state and oxygenation regime, as well as the similar trends in Chironomini $\delta^{13}C$ values we consider it highly unlikely that this is the primary cause for changes in ephippia $\delta^{13}C$ values in the sediments of Lake de Waay.

Conclusions

We have shown that in the currently hypertrophic Lake De Waay CH_4-derived carbon plays a relevant role in the pelagic food web and most likely also in parts of the benthic food web, based on the very low $\delta^{13}C$ values of *Daphnia*, floating *Daphnia* ephippia and some chironomids in comparison to POM values and sedimentary organic matter. This is clearest in fall (Figure 2), when photosynthetic primary productivity as food source is declining, and stored hypolimnetic CH_4 is mixed with oxygen-rich water layers, providing favourable conditions for MOB. Our down-core study revealed that during the beginning of the 20th

century higher nutrient levels and relatively lower oxygen availability occurred, comparable to the modern situation (Figure 3). Under these conditions $\delta^{13}C$ values of remains of *Daphnia* and Chironomini were very low, and lower than may be expected from feeding on photoautotrophic biomass only [11–15]. This suggests a significant contribution of CH_4-derived carbon to the lake´s food web, comparable to the modern situation. In contrast, the more mesotrophic phase between ca. 1925 and 1955, which was associated with higher oxygen availability at the sediment-water interface, was associated with distinctly (up to 8 ‰) higher $\delta^{13}C$ values in the investigated invertebrate remains (Figure 3).

We conclude that the eutrophication of the lake resulted in an increase in primary productivity and an increase in strength and duration of hypoxic conditions, which allow for both increased CH_4 production in the sediment and increased build-up of dissolved CH_4 in the hypolimnion [4–6]. MOB can be expected to thrive under these conditions and can therefore provide a more readily available food source for *Daphnia* in the water column and Chironomini living in sediments near the oxycline. This implies that even though eutrophication can lead to a higher availability of algal organic matter in lakes, some invertebrate taxa may benefit from the higher availability of CH_4-derived carbon as an alternative food source, leading to an increased role of CH_4-derived carbon for at least some sections of the lake food webs, and that oligotrophication can have the opposite effect. It is likely that the increased CH_4-derived carbon utilisation is due to higher CH_4 production (in addition to a longer build-up of hypolimnetic CH_4)[6], which implies that CH_4 emissions by lakes are potentially higher after eutrophication events and that (re-)oligotrophication may lower CH_4 emissions. This is also confirmed by experiments that revealed increased methanogenesis in sediments after addition of both P and N [56]. Based on our record there may be multiannual to decadal-scale lags between variations in nutrient concentrations and changes in CH_4-derived carbon entering lake food webs.

Data

The down-core stable isotope data as well as the DI-TP reconstruction can be found in the supplementary material in comma-delimited text format.

Competing interests

We have no competing interests.

Author´s contributions

The study was designed by JS, MvH and OH. The field work was carried out by JS and MvH. Processing samples for isotope analysis was done by JS. PB performed the microbiological analyses; EK performed the diatom analysis; AL was responsible for the dating of sediments and ML for stable isotope analysis of the gas samples. All authors helped draft the MS and gave final approval for publication.

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560 **Figure 1:** Bathymetric map of Lake De Waay. The circles indicate the coring locations (see
561 supplementary material): (09) core WAY09 presented in this study, (05) core WAY05
562 investigated by Kirilova et al. (2010b) from which we used the XRF data (see below).
563 Asterisks (*) indicate locations of the surface sediment samples at 2.5, 5, 8 and 14.5 m depth.
564 Lake water nutrient concentrations and pH were measured in August 2011.

565

566 **Figure 2:** $\delta^{13}C$ values of (a) POM, (b) Chironomini body tissue, (c) *Daphnia* body tissue
567 (open circles) and *Daphnia* ephippia (dots) sampled on September 1 and December 1 2009
568 and March 1 and June 1 2010. The lines indicate the $\delta^{13}C$ values of bulk sediment organic
569 matter from the top sample of the core (a), $\delta^{13}C$ values of Chironomini head capsules from
570 surface sediment at 5 m water depth (two replicates) (b), and $\delta^{13}C$ values of *Daphnia* ephippia
571 from surface sediments at 5 m water depth (2 replicates, dashed lines) and 15 m water depth
572 (solid line) (c). (d) Boxplot showing the range of $\delta^{13}C$ values from the field survey, as well as
573 the $\delta^{13}C$ of CH_4 sampled in the sediment and in oxic and anoxic water. Whiskers of the
574 boxplots encompass data points no more than 1.5 times the interquartile range from the box.

575

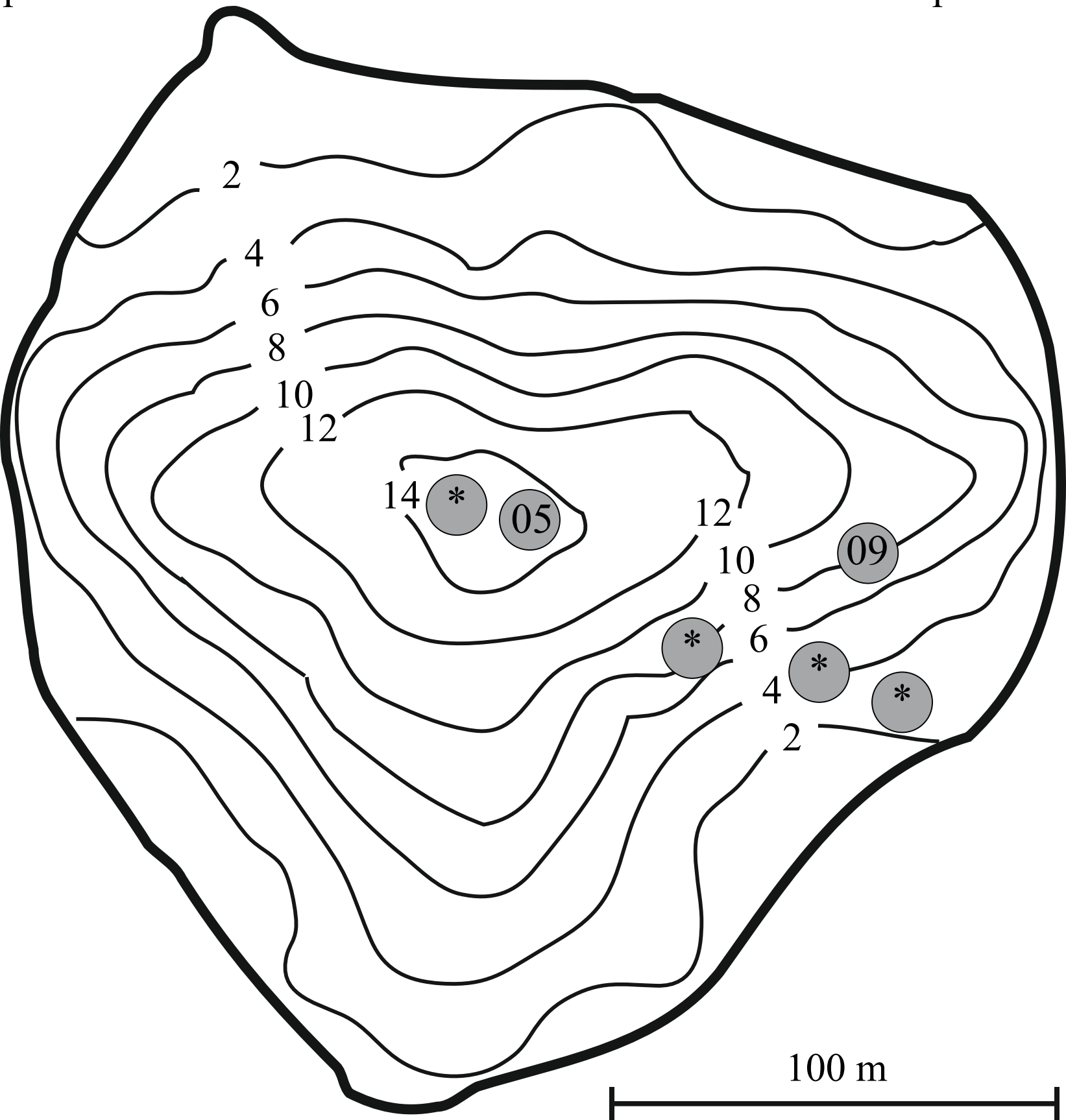
576 **Figure 3**: Diatom-inferred TP (DI-TP) for the De Waay sediment record, grey lines indicate
577 estimated standard errors of prediction (note the log scale); Fe : Mn of the sediments (grey
578 line: high resolution measurements (near-yearly resolution), black symbols: average values
579 for intervals represented by fossil samples); $\delta^{13}C$ values of bulk sediment organic matter
580 (closed triangles), Chironomini head capsules (closed diamonds) and *Daphnia* ephippia
581 (closed circles) (note inverted scale); and the estimated contribution of CH_4-derived carbon in
582 the diet of Chironomini (open diamonds) and *Daphnia* (open circles) based on a two-source
583 mixing model. Fe : Mn ratios are from core WAY05 taken by Kirilova et al. [34] in the lake

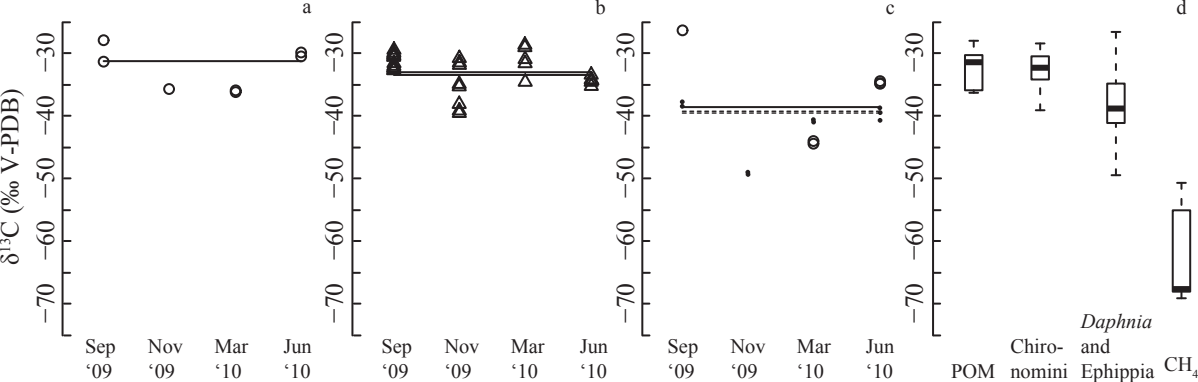
584 centre, and DI-TP and $\delta^{13}C$ data from core WAY09 obtained from 8 m water depth (this

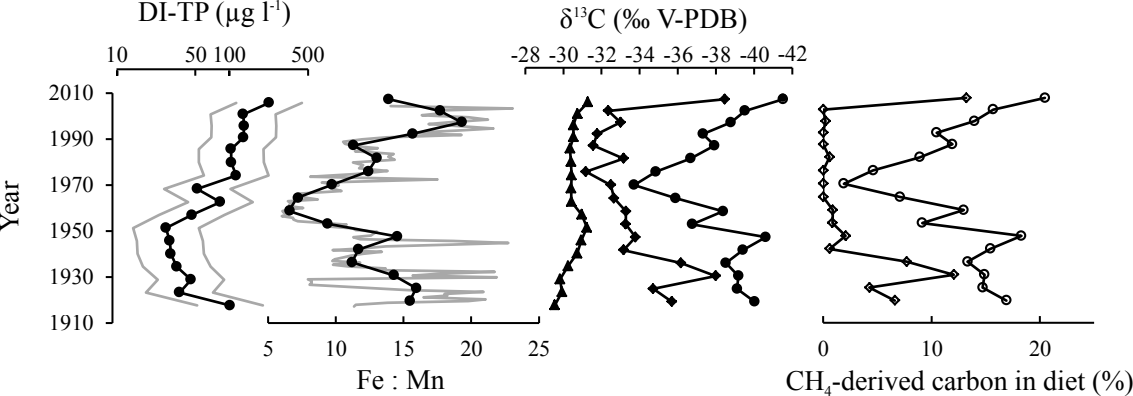
585 study).

Surface water
TP 120 µg l^{-1},
TN 2300 µg l^{-1}
pH 8.0

Bottom water
TP 1500 µg l^{-1}
TN 3600 µg l^{-1}
pH 6.9




a
b
c
d
δ13C (‰ V-PDB)
−30
−40
−50
−60
−70
Sep '09
Nov '09
Mar '10
Jun '10
POM
Chiro-nomini
Daphnia and Ephippia
CH4


DI-TP (µg l-1)
10
50 100
500
δ13C (‰ V-PDB)
-28 -30 -32 -34 -36 -38 -40 -42
Year
2010
1990
1970
1950
1930
1910
5
10
15
20
25
Fe : Mn
0
10
20
CH4-derived carbon in diet (%)